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(54) PROCESS FOR THE PRODUCTION OF LACTULOSE AND FOODSTUFFS CONTAINING LACTULOSE

(71) We, HAYASHIBARA COMPANY, a Body Corporate organised and existing under the laws of Japan, of 2—3, 1-chome, Shimoishii, Okayama-shi, Okayama, Japan, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a process for the production of lactulose from lactose and, more particularly, to a process for isomerizing lactose to lactulose using a basic catalyst, converting the residual lactose into the corresponding aldonic acid by oxidation with lactose dehydrogenase, and optionally thereafter removing the acid.

No natural lactulose exists. In 1930, Montgomery succeeded in synthesising lactulose by isomerizing lactose using Lobry de Bruyn's reaction. In this method the separation of the residual lactose requires the concentration of the reaction solution, crystallization of lactose by the addition of methanol and separation of the crystallized lactose; then removal of aldose from the residual solution by oxidation with bromine. However this method was too complicated and commercially unviable from the industrial stand point. The method requires the following steps which make it complicated and unapplicable for industrial production. First expensive bromine is used; secondly the removal of bromine from the solution is carried out using silver sulphate; thirdly the removal of the residual silver is carried out with sulphorous acid; and fourthly ketose is first obtained by extraction with methyl alcohol of the resultant solution. The yield using Montgomery's method is only 15% and attempts to isomerize lactose using sodium hydroxide or ammonium hydroxide which have been made, gave similar yields of approximately ten per cent. R. Kuhn *et al* produced lactulose from lactose via N - (p - tolyl)-lactosylamine using the Amadori reaction. However the yield using this method is also approximately ten per cent and in addition the process is more expensive.

Our studies and efforts have resulted in an industrially feasible process for the production *inter alia* of pure lactulose.

Thus according to the present invention there is provided a process for the production of a mixture of lactulose and lactobionic acid, comprising isomerizing lactose to lactulose with a basic catalyst, oxidising the unreacted lactose in the resulting reaction solution to lactobionic acid by cultivating a lactose dehydrogenase producing strain of the genus *Pseudomonas* on the reaction solution, or by adding to the reaction solution lactose dehydrogenase containing cells of such a strain.

Thus according to the invention pure lactulose is obtainable by isomerization with basic substance and enzymatic separation of the unreacted lactose with oxidation with lactose dehydrogenase followed for example by ion exchange purification or sedimentation of the oxidation products for example in the form of calcium salts.

The reaction mixture which is obtained after the isomerization stage leaves more than half of the original lactose. Therefore separation of the coexisting lactose has a significance from the point of industrialization. Lactose crystallizes easily because of its low solubility. Taking advantage of this property, separation of most of the existing lactose is possible by crystalization upon concentration. Subsequently the still remaining lactose is oxidised enzymatically using the specific enzymatic reaction, into the corresponding bionic acid (lactobionic acid), thus changing greatly the properties of the aldose. Subsequent separation can be performed easily owing to the fact that lactulose remains intact.

Screening of various strains belonging to the genus *Pseudomonas* to find the suitable strains indicated that lactose dehydrogenase produced by *Pseudomonas graveolens* or *Pseudomonos*

fragi was most suitable for this purpose. Evidence was presented that this enzyme acted fully on aldoses, especially lactose, converting them into the corresponding bionic acids. However no reports are available on effects of the said enzyme on lactulose. We performed cultivations with strains of the genus *Pseudomonas* on media comprising aldose or ketose and found that on lactose and lactulose, strains of *Pseudomonas graveolens* and *Pseudomonas fragi* were effective, and further observed that the enzymes reacted 100% only on aldose without any reactions on ketose. However it was also found that other ketoses such as maltulose were effected by some bacteria. In addition when the intact cells obtained from such cultures are used as an enzyme source to perform the oxidation reaction, identical results were obtained.

The amounts of inorganic and organic compounds to be added during cultivation of the said strains are extremely small and removal of these additives after reaction can be carried out with ease. Calcium added for adjustment of pH forms calcium bionate which is removed intact subsequently.

The resulting bionic acid consists of the basic calcium salt which can be separated easily by precipitation or can be separated by adsorption on basic ion exchange resin after the metallic ions are removed with ion exchange resin. Furthermore the bionic acid is more completely separated by combining both methods. And as bionic acid is obtainable in the state of metallic salt or free acid, the ketose is collected simultaneously with bionic acid thus increasing the economic value of the present invention.

Owing to the fact that lactose is commercially available in a very pure state, being produced from milk whey at a purity of over 99%, bionic acid and ketose produced according to this invention have uniform purities.

The isomerization of lactose to lactulose using a basic catalyst is generally known as the Lobry de Bruyn reaction, and has for many years been the simplest method of isomerization of lactose into lactulose. Isomerization proceeds by heating strong basic aqueous solutions such as of calcium hydroxide, potassium hydroxide, sodium hydroxide and ammonium hydroxide or by contacting sugar solutions with strong basic anion ion exchange resin. However sugars are unstable at a high level of alkalinity, especially at high temperatures, and oxidative degradation rapidly occurs. However, any of the above catalysts is employable and a wide range of variation of temperature and reaction period, which depends on production equipment and other conditions, can be made; thus a high temperature and short reaction period or low temperature and long reaction period can be selected. As the earlier methods were performed with low temperature and long period reactions which caused comparatively low decomposition, we performed tests on high temperature short period reactions as well as on low temperature, long reaction periods. In the case of high temperature reaction oxidative degradation varied depending on the amount of alkali used and temperature. In order to prevent contact with air and to effect heating within a short period, the mixture was passed at a constant rate through a heated pipe, cooled and neutralized. The results are listed in the following table.

TABLE

$Ca(OH)_2$ Concentration 1.5% vs. lactose concentration 20%

Heating temperature \ Heating time	Isomerization degree per raw material, Degradation loss (%)			
	5 min.	10 min.	15 min.	3 days
35°C				43% (1%)
80°C		36% (1.5%)		
100°C	30% (1.5%)		45% (4%)	

Sugar concentration in the range 20—40% were found suitable. Though low temperatures were preferred, reactions carried at high temperatures, of over 80°C, made avoidance of sugar loss and treatment within the extremely short period of a few minutes possible.

In the case of low temperature reactions calcium hydroxide was employed, and 0.13% solutions were kept standing for 3 to 4 days. Identical results were obtained using sodium hydroxide or ammoniu hydroxide. High temperature reactions were performed at an alkalinity of 1—2%, pH 9—10. In this case sugar loss was retained at 1—2% by short period treatment. Though a vivid colouration was observed, an isomerization rate of 30—40% was obtained. The iomerization rate was analyzed by Cysteine - H_2SO_4 - carbazole method. The resultant isomerized sugar was cooled rapidly and neutralized by charging into a flash tank, maintained at reduced pressure, in which the reaction mixture was neutralized with dilute hydrochloric acid or dilute sulphuric acid. Calcium sulphate thus formed was removed by filtration. Sodium chloride, ammonium sulphate were employed intact in the subsequent cultivation or enzymatic reaction. The coloured reaction mixture was decolourized with a granular decolourizing carbon layer. Analysis showed that formation of lactulose was nearly 40%, the presence of small amounts of decomposition products, besides unreacted lactose were observed in higher Rf points.

When the resulting solution was treated with ion exchange resin a syrup with a stronger sweetness than lactose and with an applicability as a sweetener was obtained. Separation of lactose which crystallized upon concentration increases the sweetness of the syrup which comprises over 70% lactulose.

Strong basic ion exchange resins can be used as basic solid catalyst although a longer reaction period and a higher temperature are required. This method therefore has the following disadvantages: necessity of stirring the solution in the absence of air in order to prevent oxidation of sugars, decrease of alkalinity of ion exchanger caused by the acid formed, and decrease of run cycles even when comparatively high thermo-resistant resins are used. A suitable resin is Amberlite IR 400 (AMBERLITE is a Registered Trade Mark). Upon circulation at 70°C for over 5 hours an isomerization degree of over 30% was obtained from the 30% sugar solution free from air.

Separation of the isomerized sugar solution, i.e. aldose and ketose mixture, was fully accomplished through enzymatic oxidation and subsequent processing. Oxidation of the residual aldose into aldonic acid is carried out by cultivation on the isomerized sugar containing solution as carbon source of *Pseudomonas* strains which produce aldose dehydrogenase, or by enzymatic dehydrogenation of the isomerized sugar solution. More particularly, when strains of the genus *Pseudomonas* are culture with aeration on a 5—10% isomerized sugar solution containing a nitrogen source, such as corn steep liquor and of a small amount of inorganic salt, the ketose is hardly attacked whereas the aldose is oxidized into aldonic acid, i.e. lactobionic acid.

Cells obtained from the aforesaid culture broth can be added to a 20—30% isomerized sugar solution. By oxidation with aeration and agitation, while preventing a decrease in the pH value by the use of a basic substance, such as calcium carbonate, the residual lactose can be oxidized, similarly, into aldonic acid resulting in little loss of ketose. The strains that produce dehydrogenase most effectively without any hydrolysis of biose were *Pseudomonas græveolens* (IFO 3460) and *Pseudomonas fragi* (NRRL 25). The reaction period required was 10—20 hours. The degree of oxidation was determined by quantitative analysis of calcium aldonate.

Pseudomonas græveolens IFO 3460 is described in the Specification of Application No. 27387/70 (Serial No. 1,320165), and *Pseudo-*Biol Chem, vol. 171 (1947), pp. 213—221.

From the reaction products i.e. a mixture of aldonic acid and ketose, calcium can be removed by a strong acidic resin. Utilizing the acidity of aldonic acid, subsequent separation of ketose is possible for example by absorption of the acid on basic ion exchange resin. Another possible method of separation is crystallization into basic calcium salts of aldonic acid. That is, after concentration the mixture was made slightly alkaline by adding thereto calcium hydroxide, and aldonic acid was precipitated as a basic calcium salt. The formed crystals were decomposed with sulphuric acid. To the solution from which the formed calcium sulphate was removed, was added a small amount of barium salt to remove the remaining sulphate ions. Upon removal of the residual metallic ions by a cation exchanger, bionic acid was collected. The mother liquor, from which aldonic acid was removed, was purified with acidic and basic resins to obtain pure lactulose.

In order to remove aldonic acid directly with ion exchange resin, after removal of calcium ion with strong acidic resin, aldonic acid was absorbed on weak basic ion exchange resin and eluted with strong alkali. The salts of aldonic acid in the eluate were purified with strong acidic ion exchange resin to obtain free aldonic acid.

Further, by purification with active carbon and strong acidic ion exchange resin, the ketose and aldonic acid mixture became a sour treated sweetener which is applicable *per se* as a food additive.

Lactulose has a refined sweetness. Sweetness of conventional sweeteners such as glu-

cose, fructose, bee honey and starch syrup for example and especially sucrose is excessive, stimulative and monotonous, but not mild. High concentration of these sweeteners increases the tendency to crystallization. Also all of these metobolised and have a high calorie content value. Recently demands for sweeteners with superior, refined and retentive sweetness and flavour and for unmetabolised food additives with non or low calorific values have increased greatly in the pharmaceutical industry and amongst figure conscious people generally.

Though lactulose, 4 - O - β - D - galactopyranosyl - α=D - fructose, has similar physical properties as cane sugar which is a disaccharide, its sweetness is extremely mild. The sweetness of lactulose is not stronger and not stimulative as in the case of cane sugar. Lactulose is inert to enzymatic hydrolysis, is an attractive non-caloried sweetener, and is a desirable sweetener for the production of new types of foods and beverages. The following are the characteristics of lactulose with regard to its use for foods.

(1) When added to foods and drinks it does not impart to the products a strong and undesirable sweetness as is the case when cane sugar is added. When it is used in conjunction with cane sugar for production of foods and beverages, lactulose softens the stimulating sweetness of cane sugar and thus provides food products with well blended and refined sweetness.

(2) Since it has a molecular structure similar to cane sugar, when it is employed to produce foods and drinks, the products will have suitable viscosity and lustre. That is, by using of lactulose production of foods and drinks are possible without unsuitably low viscosity as is the case when glucose or fructose is used. Beverages with suitable viscosity and various foods such as canned goods, syrups and bean paste, with excellent appearance and appealing qualities are thus obtainable.

(3) When foods and drinks are highly sweetened with lactulose, neither crystallization nor turbidity caused by crystallization occurs as is the case when sucrose or glucose is used as sweeteners. When lactulose is used conjointly with sugar or glucose crystallizations of these sugars are prevented and foods and drinks with prolonged shelf life are available.

(4) Lactulose has the structure of a ketose which is identical with fructose and has flavour and moisture retentive properties. Thus lactulose provides beverages and food such as canned foods and cakes with a stabilized flavour and sweetness.

(5) As the sweetener is inert to hydrolysis by amylase and is not absorbed in animal bodies, it is a non-calorific, body rendering and sweet substance. Foods and beverages that contain this sweetener can be defined as non-calorific products.

The obtained products have the following properties:

(1) *Sweetness*

Results of panel tests on sweetness show that the sweetness of lactulose is weaker than that of sucrose but identical with that of glucose. Its sweetness quality is milder and more refined when compared with sucrose.

Panel tests were conducted by thirty examiners. Tests on sweetness were carried out by paired preference tests with sucrose, glucose and lactulose. Comparisons were repeated more than five times with each substance. Results of comparisons at concentration degrees of 35%, 20% and 10% gave the following order:

Sucros > lactulose = glucose > lactose

(2) *Hardly crystallizing property and crystallization preventive property*

The lactulose was freely soluble in water. Even at high concentration lactulose was difficult to crystallize. Furthermore no crystallization was observed in the cases where 10% of lactulose was added to 70% concentration of sucrose and glucose respectively. Solution of sucrose or glucose without lactulose crystallized within a short period.

(3) *Non-calorific property*

1. *Inertness to enzymatic hydrolysis Reaction Conditions*

Temperature: 40°C

pH: (A) (B) (C)
5.0 6.0 7.5

Reaction, substrate: final 1% 5 ml

0.1 M buffer solution: (A), (B) acetate 4 ml
(C) Mc Ilvane

Enzyme solution: 1 ml

Hydrolysis was measured by determination of the increase of reducing sugar. Reducing and total sugars were determined by Schoorl's method, directly, and after hydrolysis for an hour at 100°C with 1 ml of 25% HCl and neutralization, respectively.

(A) Lactose of which the decomposition rate was defined as a control by the effects of *Rhyzops* and glucoamylases was decomposed 100% in 5 hours, whereas no decomposition of lactulose was observed.

(B) Decomposition rate effected by invertase. Cane sugar was decomposed completely in 15 minutes, whereas lactulose showed no evidence of decomposition even after a reaction period of 120 hours.

(C) Decomposition by extracts of pig pancreas.

Maltose was decomposed completely after 26 hours, while no decomposition was observed in the case of lactulose.

2. *Tests on animals*

Absorption tests within digestive organs of higher animals showed that lactulose was totally unabsorbed and completely non-calorific. Into the intestines, both ends-tied, of live rabbits which had been fasted for 24 hours were injected 50 ml of a 20% lactulose solution and 50 ml of a cane sugar solution. Comparison results showed that 90% of the cane sugar was absorbed within several hours, while in the case of lactulose no indication was observed which indicated that lactulose was absorbed. Observation on intestine walls was normal.

(4) *Moisture and flavour retentive properties and viscosity*

The sweetener is a ketose with a molecular weight identical to cane sugar. It is superior in respect of its moisture and flavour retentive properties and viscosity. It is especially advantageous in the production of Castella and sponge cake for example because of its effectiveness in rendering a damp and soft texture to these products. This sweetener imparts desirable viscosity to canned fruits and fruit juice and its moisture and flavour retentive properties are effective in rendering a fresh flavour and taste and a unique palatability to food products.

(5) *Stability at various pH levels*

Final sugar concentration	0.02%
Final 0.02 M buffer solution, pH	2.0—10.0
Temperature	98°C

Samples were taken at intervals. After determining changes of reducing sugars and total sugar by the Somogyi Nelson method the initial concentration was expressed as 100.

To 1 ml reaction solution was added 0.5 ml of 5% HCl. The mixture was incubated at 100°C for 45 minutes and neutralized with 0.5 ml of a 5% aqueous NaOH solution. Determination of total sugar was made by the Somogyi Nelson method and the results were expressed as the amount of total sugar. The results are given in the following table.

pH			Heating time				
initial	after 12 hours		1	2.5	5	8	12
2.0	2.3	Reducing sugar	100	102	103	107	110
		Total sugar	—	100	101	102	103
4.0	4.0	Reducing sugar	101	101	99.5	100	100
		Total sugar	—	100	—	100	102
6.0	6.5	Reducing sugar	100	100	98.0	99.0	99.5
		Total sugar	—	102	—	98.0	97.8
9.0	8.2	Reducing sugar	105	104	103	104	103
		Total sugar	—	91.3	84.0	80.0	75.3

Though in the pH 3.0—8.0 it was safer to treat the solution at 98°C for 12 hours, at pH 2 an extremely small amount of lactulose undergoes hydrolysis, while at pH 9.0 sugar itself had a tendency of decomposition.

As above described, as a sweetener lactulose is most suitable for various refreshing beverages. Therefore in carbonated beverages such as cola drinks, ciders and lemonade lactulose blends with the sourness of carbonic acid, refreshing the after-taste. When employed in lactic drinks such as Calpis and yogurt, lac-

tulose harmonizes well with lactic acid, improves its taste and retains its flavour. Also the use of lactulose in natural fruit juice, artificial fruit juice and concentrated fruit juice for example helps retention of the fresh fruit flavour and imparts a refreshing sweetness to the products.

Addition of lactulose in small quantities to alcoholic drinks such as beer greatly improves the product's flavour. Also addition of lactulose into highly concentrated juice causes no crystallization of the sweetener and thus products can be maintained at high sugar contents. Especially in cases where the use of artificial sweetener is undesirable, the effectiveness of lactulose is significant.

The mild sweetness and strong flavour retentive properties of lactulose give good results to ice cream, ice candies, sherbet and other frozen desserts and also to canned fruits and tomato kechup for example. Lactulose is also effective in the production of ice cream because lactulose has the advantages of maintaining higher melting points, of increasing over run and of imparting softness to the products.

Most notable is its non-calorific value which makes the production of completely non-calorific beverages possible, this has significance in production of pharmaceutical products and food and beverages for weight or figure conscious people.

Lactulose is mose effective in Western style and Japanese style confectionery because of its refined sweetness, moisture retention and its tendency not to crystallize. Various cakes prepared with lactulose show no degradation with drying and crystallization of the sweetener. It also renders to the products a moist texture with desirable dampness. As a result of the products' non-calorific value, processes for low caloried foods are provided by substituting half (by weight) of the normal ingredient sugar with the lactulose. Lactulose, when added in amount of a few percent by weight to breads and bakery products, remains undecomposed in the final products. Lactulose imparts a refined sweetness, flavour, colour and texture to the products.

In bakery goods, for example, biscuits and cookies, the use of the sweetener provides most desirable finished baked colours to the goods. The moisture retention of lactulose minimizes deformation or breaking and also improves the yield of these products.

Also when used for production of jellies, lactulose imparts a non-caloried and refined sweetness to the products, maintains flavour, stabilizes colour, prolongs transparency of the products in a preferable state and does not cause excessive drying or plasmolysis and thus maintains constantly the initial freshness of the products.

Further, when lactulose is employed in various creams as a sweetener, it imparts a soft sweetness and soft palatability to the products.

Moreover incorporations of lactulose to hard candies, chocolate ball centres, chewing gums and caramels for example moderate the conventional excessive sweetness, increase and improve the aftertaste and are effective in modernization of tastes. In addition it lowers the calorific values of the products.

Addition of a small amount of lactulose to Japanese or Western style alcoholic drinks greatly improves the flavours and increases the body of drinks.

As described above, because the use of lactulose as it is, wherein aldonic acid is present, for foods and drinks, imparts to the products flavour and moisture retentive and crystallization preventive properties and also imparts mild sourness, lactulose is a useful food additive which is effective in imparting natural sweetness and sourness to foods and drinks, such as wine, jellies, soft drinks, canned fruit, frozen desserts, mayonnaise, pickles, chewing gums and hard candies for example.

The invention will now be further described with reference to the following Examples in which all parts and percentages are by weight (d.s.b.):—

EXAMPLE 1.

1 kg of lactose was dissolved in 5 litres of water. Upon adding 6 g calcium oxide, the solution was incubated at 35°C for 4 days. The optical rotation of the reaction solution became almost constant. The reaction mixture was concentrated under reduced pressure, decolourized and then purified with active carbon. The lactose crystals which formed were filtered and washed. The mother liquor from which the crystallized lactone was separated, was combined with the washings. The resulting solution was adjusted to give a sugar concentration of 10% and was then neutralized with carbon dioxide. After the addition of 1% corn steep liquor, 0.2% urea, 0.05% KH_2PO_4 and 0.025% $MgSO_4.7H_2O$ to this solution, the solution was autoclaved and to the solution was added 2.5% calcium carbonate which had been sterilized separated. *Pseudomonas graveolens* (IFO 3460) was inoculated and cultivation was performed at 30°C for 50 hours with air equivalent perminate and stirring at 400 r.p.m. Cells were collected from the culture broth by centrifugation and were employed in the oxidation of lactose as a dehydrogenase source in the subsequent tests.

Upon completion of the cultivation the culture broth from which cells were removed, was decolourized, filtered and concentrated and its pH was brought to slightly alkaline by the addition of saturated calcium hydroxide. The formed basic calcium salt of bionic acid was filtered and bionic acid was obtained therefrom eventually. The filtered liquor was saturated with carbon dioxide to remove calcium ion therefrom as much as possible. The solution was decolourized by passage through ion exchange resin (Triple bed system: Amber-

lite IR 120, IRA 69, IR 120+IR 411) and was concentrated to a colourless solution. Results of analysis showed that the lactulose had a purity of 99% and thus contained only an extremely small amount of lactose. The yield was 40% of the lactose used.

The precipitate in the form of insoluble calcium salt which was separated from the lactulose was suspended in 3 times its weight of water. Dilute sulphuric acid was slowly added. Calcium, precipitated in the form of calcium sulphate, was removed by filtration. The solution was passed through a strong acidic resin (Amberlite IR 120) to remove residual ions. A minimum amount of basic resin to effect adsorption of all the IR 400 in the solution was added and the resins on which the sulphuric ions were adsorber were removed. Concentrations in vacuum was performed to obtain lactobionic acid in a syrup state. Paperchromatographic tests showed that the product consisted of 98% bionic-acid and small amounts of lactose, lactulose and others. The yield was 13% of lactose used.

EXAMPLE 2.

To a 1 litre of a 15% lactose aqueous solution was added 200 ml ammonium solution (specific gravity 0.88). The solution was kept at 35°C for about 4 days till optical rotation became constant. After neutralization to pH 5.8 with sulphuric acid, 0.5% corn steep liquor, 0.06% KH_2PO_4, 0.025% $MgSO_4.7H_2O$ were added and the mixture sterilized. *Pseudomonas fragi* (NRRL 25) was inoculated on the mixture and cultivation was performed with aeration and adjusting its pH with calcium carbonate as shown in Example 1. After removal of the cells, calcium carbonate and calcium sulphate, the culture broth was concentrated and concentrated lime water was added to make the broth slightly alkaline in order to crystallize bionic acid as its basic calcium salt. The formed precipitate was separated by centrifugation and washed with a small amount of water. The supernatant and washings were mixed together. From the solution were removed metallic ions and residual aldonic acid by purification using a triple bed system ion exchange unit. Thus a slightly yellow syrup of lactulose was obtained in a yield of 38% based on the lactose used as anhydrous substance.

To one part of the lactulose thus separated and concentrated to 85% were added two parts of warm methanol and the mixture can then cooled. Cooling was conducted by gentle stirring while adding crystal seeds. The crystals which formed were filtered and thus pure lactulose was obtained.

The calcium components present in basic calcium salts of lactobionic acid were removed as sulphates, following the method described in Example 1. Subsequently deionization was performed with ion exchange resin to obtain aldonic acid syrup. The yield was 47% of lactose. Paper chromatography determination showed that no other organic acids were present.

EXAMPLE 3.

Two litres of a 15% lactose aqueous solution to which 2 g calcium oxide was added, was reacted at 35°C for 3 to 4 days. Upon conclusion of reaction, neutralization with carbon dioxide and sufficient decolouration with active carbon, were carried out and to 1 litre of this solution was added 37 g calcium carbonate, and 5 g of the enzyme containing cells obtained in Examples 1. The mixture was aerated and stirred vigorously at 30°C for 15 hours whereupon the reaction was stopped. After decolouration and purification, metallic ions were removed by passing the mixture through Amberlite IR 120. Adsorption of aldonic acid using a weak basic ion exchange resin, Amberlite IRA 69, was carried out. A colourless lactulose solution was obtained by passing the thus purified mixture through a mixed bed of IR 120 and IRA 411. The concentrated product was pure lactulose containing no lactose. The yield was 38% of lactose. Lactobionic acids adsorbed on the ion exchange resin was eluted with sodium hydroxide. The sodium bionate thus obtained was formed into free acid by passing it through a IR 120 tower. A syrup was obtained upon concentration. The yield as acid was 47% of lactose.

EXAMPLE 4.

A 30% aqueous solution of lactose was prepared and adjusted to pH 10.0 with sodium hydroxide. Using a continuous unit isomerization was carried out by heating to 95°C for 5 minutes. The reaction solution was continuously and rapidly cooled and neutralized with hydrochloric acid. After decolouration by passing the solution through a granular active carbon layer, 4 metres high, desalting was carried out with ion exchange resin. A slightly yellow syrup was obtained.

The isomerized syrup contained about 40% lactulose.

The isomerized mixture obtained was adjusted to 10% concentration. 2.5% calcium carbonate per solution was added. 200 mg of the enzyme containing cells obtained in Example 1 were added per 10 g of sugar as enzyme. The mixture was aerated and stirred at 30°C for 20 hours. The mixture wherein most of reaction of lactose disappeared, was heated and decolourized with active carbon. Subsequently after removal of metallic ions by strong acidic ion exchange resin, Amberlite IR 120, lactobionic acid was removed by adsorption on Amberlite IRA 69. The acid-free lactulose solution was purified with a mixed bed system containing both strong acidic and strong basic ion exchange resins; it was then concentrated. A colourless syrup with yield of

37% compared to the material lactose, was thus obtained. Analysis showed that the lactulose obtained contained only 1% lactose.

The lactobionic acid adsorbed on the ion exchange resin was diluted with an aqueous sodium hydroxide. After removal of sodium with IR 120 the solution was purified with active carbon and concentrated. The yield was 52% of the starting lactose. It was paper chromatographically pure.

EXAMPLE 5.

A 25% lactose aqueous solution from which air had been expelled by boiling was repeatedly circulated at 70°C through a tower packed with a large amount of IR 400 (Rhom and Haas Co.). Oxidation of sugar was prevented by maintaining the system as air free as possible. Within 5 to 7 hours the degree of isomerization reached 32%. The isomerized solution was thereafter decolourized with active carbon, and then purified using a mixed bed of Amberlite IR 120 and IR 411.

Although the sugar solution thus obtained was a syrup with a strong sweetness, the separation and purification procedures to lactulose were performed in accordance with the methods described in Example 4. The yield of lactulose and lactobionic acid were 30% and 57% based on the starting lactose respectively.

The following Examples illustrate the use of lactulose obtained in accordance with the invention in various foodstuffs.

EXAMPLE 6.

Process for the production of carbonated beverages

A formula example for production of cider, which is a carbonated beverage, is given below. (per cider 10 l)

Lactulose	700 g
Saccharine	12 g
Salt	2 g
Citric acid etc.	60 g
Flavouring	50 ml

The above ingredients were dissolved thoroughly, carbonated by a known method, and then the drink could be bottled. The sweetener harmonized desirably with the artificial sweetener and the sourness of the other materials contained in the drinks, and also imparted to the cider a refined delicious and sophisticated sweetness and a freshness. Since the sweetener is non-calorific, no substances with a calorific value are present in the resultant products, thus the products can be defined as a completely non-calorific beverages.

EXAMPLE 7.

Process for the production of concentrated fruit juice

Ingredients of 1000 l concentrated juice containing orange juice are: 79 l of orange juice concentrated to 5 times its normal strength, 750 l of 60% lactulose solution, 40 kg of citric acid, 5 l of orange base, 2.5 l of orange essence. To the above ingredients was added water to give 1000 l solution.

The lactulose sweetener emphasized the sourness and inherent taste of oranges and thus harmonized favourably with oranges. Since lactulose is a sugar which is difficult to crystallize, there is no formation of crystals even in juices concentrated several times, and thus clear concentrated juices are obtainable.

EXAMPLE 8.

Process for the production of canned mandarin oranges

Preparation of syrup to be charged into the canned mandarin oranages was carried out by the addition of water to 60 kg of the lactulose sweetener and 30 g of saccharine to form a 100 kg solution. The sarcocarps obtained by peeling the mandarin oranges and to which were added the above prepared syrups, were canned and sterilized according to the usual methods. Thus the canned mandarin orange products were obtained. By the utilization of the lactulose sweetener, the viscosity of the sweetener imparted a desirable gloss to the fruits. Its mild sweetness harmonized well with the sourness of mandarin oranges, and also emphasized and retained their flavours. Since the fairly high concentrated syrup was non-calorific, the products according to this proceess may be considered as low-caloried fruits.

EXAMPLE 9.

Process for the production of tomato ketchup

To 10 l of tomato puree prepared from tomatoes, which were crushed to pulp, strained and concentrated to 40 to 50%, were added 800 g of the lactulose sweetener, as anhydrous substance), 100 g of salt, 100 g of 30% acetic acid, 50 g of onions, 2 g of all spice, 5 g of clove oil, 12 g of cinnamon, 2 g of mace, and 1.0 g of red pepper. The mixture was concentrated to form a final product, which had a specific gravity of 1.128.

The product maintained its vivid lustre and colour. The lactulose sweetener imparts a mild sweetneess to the products, blended desirably with the tomatoes, and emphasized their fresh flavour.

EXAMPLE 10.

Process for the production of ice creams

The lactulose sweetener is suitable for ice creams, sherbets, ice cakes and other frozen desserts. For an example a process for the production of ice cream is given. 15 parts of fresh cream (fat content 45%), 30 parts of skim milk, 5 parts of lactulose sweetener and as for stabilizer 0.5 parts of carboxyl methyl cellulose (CMC) were added to 50 parts of milk. The mixture which is homogenized by

mixing and dissolving at 50 to 75°C is brought to 60%65°C and then filtered to remove the alien substance. Fats in the resultant products were blended with a blender and thus the over-run was increased. After distillation at 70°C for 30 minutes, the product was cooled and kept at 3 to 5°C. The final product was obtained upon ageing and freezing. The product had a refined sweetness preferable for frozen desserts, a relatively high melting point, an improved over-run property which are all comparatible to the same properties as when sucrose is used. Thus products with high palatibility and flavour are provided.

EXAMPLE 11.

Process for the production of sponge cakes ('Castella')

Sponge cakes are products of mixtures which comprise sugar, eggs and flour. The sugar used determines the tastes and textures of sponge cakes. A typical formula for the production of sponge cakes with using sweetener of the invention is as follows. Dough is prepared according to the usual method by mixing thoroughly 100 g of lactulose sweetener, 1000 g of eggs, 500 g of flour and 50 g of honey. The dough is poured on iron plates covered with thin sheets of papers and baked in an oven at 180 to 190°C to a desirable colour. Ketose of the said sweetener imparts an excellent baked colour. The textures of the products are of fine and uniformed grain with desirable elasticity. The dampness of the products can be maintained for prolonged periods and thus the original soft texture is preserved. Complete prevention of degradation and drying are attainable and the addition of lactulose sweetener improves more favourably and more effectively the flavour of the products than when rice syrup or honey or other sweeteners are used.

EXAMPLE 12.

Process for the production of biscuits

As an example, a process for the production of soft biscuit, will be described. 100 parts of cake flour, 6 parts of starch, 40 parts of lactulose sweetener, 15 parts of condensed milk, 10 parts of butter, 10 parts of shortening oil, 8 parts of egg, 0.3 parts of salt, and 0.5 parts of baking powder were mixed in this order into an appropriate amount of water and kneaded in a mixer. Mixing was stopped immediately upon completion of mixing and thus the mixture had a hardness equal to lobes. The resultant was passed into an oven via a stamping machine. Keeping the temperatures at the entrance and exit of the oven at 270°C and 130°C respectively, the baking was conducted within 10 minutes. The products had a uniform gloss, colour and shape and also slightly dry textures. In addition the products had a uniform smoothness that has tender texture and was easily dissolved with saliva was attainable. The product had a light but not a tedious sweetness. Moreover, since the sweetener used is non-calorific the biscuits thus obtained are low caloried products.

EXAMPLE 13.

Process for the production of jellies

A formula for strawberry jelly is shown hereunder. 80.0 kg of 80 lactose aqueous solution, 11.4 kg of concentrated fruit juice (concentrated to 1/10 by volume, and containing 0.121 kg of citric acid), 10.0 kg of 4% pectin aqueous solution were used. Hot syrup containing the lactulose sweetener was prepared and at a certain temperature pectin solution and fruit juice from which pectin had been removed were added, the solution was then maintained at 80°C and the pH adjusted to make the pH of jelly 3.1. Subsequently the final product was obtained by evaporation under reduced pressure at 103°C. By conventional methods the gelatinization of products which contain less than 50% of sugar is impossible and, in the case when sucrose is used, the products tend to have excessive sweetness. Compared to sucrose, the sweetener has a mild sweetness.

Further, because the sweetener is non-calorific, there is no necessity to decreasing the amount of sugar to be added, which normally has to be done due to the fact that pectin, the main component of jellies, is converted to low methoxyl pectin. Therefore the sweetener can be applied sufficiently and thus jellies with values are obtainable.

Decomposition of lactulose by acids hardly occurs. No phenomenon of plasmolysis caused by acids is observed; the use of the sweetener thus provides desirable jellies.

EXAMPLE 14.

Process for the production of butter creams

1100 g of the sweetener (comprising 95% anhydrous lactulose and 5% anhydrous lactose) was dissolved in a suitable amount of water. The solution was condensed to a syrup state. Upon cooling the syrup is poured slowly in fat (100 ml shortening oil), which was stirred in a mixer. When the mixture had been brought to a smooth state with glass and colour, small amounts of flavour and alcoholic drinks were added and the final product was obtained. The product had a very smooth palatability with a mild sweetness. The butter cream thus produced resulted in a good blend when used in the decoration of cakes for example.

EXAMPLE 15.

Process for the production of custard cream

199 g of lactulose sweetener and 8 g of salt were mixed in 1000 g of corn starch and then 2800 g of eggs were added and stirred. To the product was added slowly 10000 ml of fresh milk with stirring. After the addition, with stirring, and gentle heating, the starch

gelatinized completely. When the resultant became opaque, the mixture was cooled and flavouring was added. The custard cream thus obtained had no excessive sweetener and retained its stabilized smoothness.

EXAMPLE 16.

Process for the production of hard candies

A process for sugar drops is given hereunder as an example of a process for producing hard candies. 60% of granulated sugar and 20% of corn syrups were dissolved in water and to the resultant mixture was added 20% of the said lactulose sweetener. The syrup thus obtained was condensed at 130°C in a vacuum pan to a moisture content of 1 to 1.5%. Then the resultant was cooled and to it were added and mixed thoroughly 0.8% citric acid, and a suitable amount of colouring and flavouring. At about 80°C the mixture was passed through a roller and screened, thus the final products are obtained. The sweetener lightened the strong sweeteness of the sucrose. Moreover it improved the clarity and qualities of the products by imparting to them a specific and a more refined sweetness. Also crystallizaof the products was preventable.

EXAMPLE 17.

Process for the production of chocolate ball centres

The product condensed as described in Example 16 was brought down to a slightly lower temperature and with flavouring, colouring, and citric acid was mixed in a kneader. The resultant was formed into grains and then cooled, or formed into balls by vacuum expansion while the material is still hot and then cooled. In each case these products were used as centres and covered with chocolate. The obtained centres have a bland taste with no excessive sweetness and blended desirably with chocolate.

EXAMPLE 18.

Process for the production of condensed milk (sweetened with sugar)

Raw milk, whose fat content has been regulated, was sterilized instantly at 110 to 130°C. Following pre-heating an amount of the lactulose sweetener equal to 15% of the raw milk, was added and the mixture was concentrated. The resultant was cooled to below 15°C and treated so that the lactose forms into microcrystals. Because of the crystallization preventive property of lactulose, crystallization of lactose was restricted to the formation of the microcrystals. A typical formula is as follows. 25% of moisture, 29% of whole milk solid, 70% of fat, 7% of protein substance, 14% of lactose, 1.5% of ash and 45% of sweetener.

The condensed milk according to this example has a creamy colour and lustre. Its viscosity is desirable since the fat is well dispersed and the lactose crystals are of microcrystal size. The texture of the product is excellent and also the product's calorific value relative to conventional condensed milk.

EXAMPLE 19.

Process for the production of chewing gums

20 of gum base was heat melted and to it was mixed and kneaded thoroughly 10 to 30% of powdered sugar, 50 to 70% of lactulose powder and 1% of flavouring. The resultant was formed in sheets of suitable thickness and shapes and dried overnight, to obtain the final products.

The sweetness of the products was of a desirable degree with with no excess but with the desirable durability. The addition of the said sweetener stabilized the flavour and refreshed the after taste of the products.

WHAT WE CLAIM IS:—

1. A process for the production of a mixture of laculose and lactotbionic acid, comprising isomerizing lactose to lactulose with a basic catalyst, oxidising the unreacted lactose in the resulting reaction solution to lactobionic acid by cultivating a lactose dehydrogenase producing strain of the genus *Pseudomonas* on the reaction solution, or by adding to the reaction solution lactose dehydrogenase containing cells of such a strain.

2. A process according to Claim 1 wherein the basic catalyst is sodium hydroxide, ammonium hydroxide or a strong basic resin.

3. A process according to Claim 1 or Claim 2 wherein calcium carbonate is added to the reaction solution or the fermentation liquor to neutralize lactobionic acid produced during the oxidation reaction.

4. A process according to any of Claims 1 to 3, wherein *Pseudomonas graveolens* (IFO 3460) or *Pseudomonas fragi* (NRRL 25) is used as the lactose dehydrogenase producing strain.

5. A process for the production of lactulose or lactobionic acid, comprising separating lactobionic acid from a mixture of lactulose and lactobionic acid, produced according to any of Claims 1 to 4.

6. A process for the production of a mixture of lactulose and lactobionic acid according to Claim 1, substantially as hereinbefore described.

7. A process for the production of a mixture of lactulose and lactobionic acid, substantially as hereinbefore described with reference to any one of Examples 1 to 5.

8. A process for the production of lactulose according to claim 5, substantially as hereinbefore described.

9. A process for the production of lactobionic acid according to Claim 5 substantially as hereinbefore described.

10. A process for the production of lactulose, substantially as hereinbefore described with reference to any one of Examples 1 to 5.

11. A process for the production of lactobionic acid substantially as hereinbefore described with reference to any one of Examples 1 to 5.

12. A mixture of lactulose and lactobionic acid, produced by the process of any one of Claims 1 to 4, 6, and 7.

13. Lactulose produced by the process of any one of Claims 5, 8 and 10.

14. Lactobionic acid produced by the process of any of Claims 5, 9 and 11.

15. A process for the production of foodstuffs comprising adding to a foodstuff lactulose or a mixture of lactulose and lactobionic acid according to any one of Claims 12 or 13.

16. A process for the production of foodstuffs, substantially as hereinbefore described, with reference to any one of Examples 6 to 9.

17. Foodstuffs produced by the process of Claims 14 or Claim 15.

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